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Anti-endotoxin therapy and the management of sepsis

More than 20 years ago, it was postulated that antibodies directed against the core region of lipopolysaccharide (LPS) could recognize an epitope shared by endotoxins from pathogenic Gram-negative bacteria. Although this postulated common epitope could not be precisely characterized, lipid A has been considered as an attractive candidate since it is the most conserved component of LPS and is the toxic part of the molecule. These researches culminated recently in clinical studies performed with two monoclonal antibodies (mAbs), called E5 and HA1-1A (Centoxin), directed at lipid A. One of the mAb, HA1-1A, has been licensed in some European countries. The cost of the antibody is high and the financial impact of such treatment is likely to be considerable; this is likely to cause budgetary problems (Taylor, 1991), leading to competition for increasingly scarce medical resources. The real impact that such therapy will have on the outcome of septic patients should therefore be carefully studied, and those who might benefit should be carefully defined. Previous data in this field, together with a detailed analysis of the clinical studies using the two mAbs should provide guidance.

Despite modern technology, many questions remain unanswered concerning the characterization and the biological effects of polyclonal or monoclonal anti-core LPS antibodies. The pattern of in-vitro reactivity of these antibodies with LPS from pathogenic bacteria has not been clarified. The reactivity in ELISA tests deserves confirmation by other methods, since LPS are amphophilic substances which form aggregates, bind poorly to ELISA plates and promote non-specific sticking of immunoglobulins to LPS hydrophobic regions (Freudenberg *et al.*, 1989; Heumann *et al.*, 1991). Even with mAbs, opposite interpretations of in-vitro results have sometimes been published. For instance, whereas HA-1A was reported to specifically recognize lipid A and to have a broad cross-reactivity in ELISA (Ziegler *et al.*, 1991), some observations have suggested that its binding to lipid A might be the result of non-specific interactions with hydrophobic substances (Baumgartner, 1991). Recently, detailed studies of several anti-lipid A mAbs have revealed a lack of cross-reactivity with LPS, i.e. the attachment of the inner core to lipid A seemed to prevent the binding of anti-lipid A antibodies to the lipid A component of LPS (Lind, Kenne &

Table. Clinical studies with human J5 antiserum, immune plasma or anti-core LPS intravenous immune globulins (IVIG)

Type of preparation (reference)	Study population	No. of patients	Outcome
Therapeutic studies			
J5 antiserum (Ziegler <i>et al.</i> , 1982)	suspected Gram-negative bacteraemia and/or shock	304	reduction in mortality
J5 plasma (Girardin <i>et al.</i> , 1992)	fulminant meningococcal purpura	73	no apparent benefit
Anti- <i>E. coli</i> J5 IVIG (Calandra <i>et al.</i> , 1988)	Gram-negative septic shock	100	no benefit
Prophylactic studies			
J5 antiserum (McCutchan <i>et al.</i> , 1983)	neutropenic cancer patients & bone marrow transplant recipients	100	failure to prevent fever and Gram-negative sepsis
J5 plasma (Baumgartner <i>et al.</i> , 1985)	high-risk post-surgical patients	268	failure to prevent Gram-negative infection; prevention of shock and death due to Gram-negative infections of borderline significance*
Anti-Re LPS IVIG (A. Cometta <i>et al.</i> , unpublished)	high-risk post-surgical patients	352	no benefit

*Statistically significant only with one-tailed Fisher's tests: the P values obtained with the Chi-square test were 0.10 for the difference in the incidence of Gram-negative shock between J5 plasma (15/136 patients) and normal plasma (6/126) recipients, and 0.08 for the associated mortality (9/136 and 2/126, respectively).

Lindberg, 1991; Rietschel *et al.*, 1991). Another area of concerns is the existence of important discrepancies in experiments of cross-protection. Differences in the animal models used cannot explain all the discrepancies, since opposite results have sometimes been obtained using similar models and similar bacterial or LPS challenges. For instance, HA-1A was reported to protect experimentally challenged mice and rabbits when tested as a crude hybridoma fluid (Teng *et al.*, 1985). Using a highly purified form of this antibody, these results could not be reproduced (Baumgartner *et al.*, 1990). Finally, the inconsistent results in clinical studies using various polyclonal antibodies is puzzling (Table).

Whole human serum or plasma from volunteers after immunization with boiled *E. coli* J5 cells has been studied in the treatment of Gram-negative bacteraemia (Ziegler *et al.*, 1982) and fulminant meningococcaemia (Girardin *et al.*, 1992), and in the prophylaxis of Gram-negative systemic infections in neutropenic patients (McCutchan *et al.*, 1983) and in high risk post-surgical patients (Baumgartner *et al.*, 1985). Hyperimmune anti-core LPS intravenous globulin has been studied in

the treatment of Gram-negative septic shock (Calandra *et al.*, 1988) and in the prophylaxis of Gram-negative systemic infections in high risk surgical patients (A. Cometta *et al.*, unpublished). Only two of these six studies appeared successful (Ziegler *et al.*, 1982; Baumgartner *et al.*, 1985). However, the mechanism of protection in these two studies with J5 antiserum remains unknown, but is not attributable to anti-lipid A antibodies since the levels of these antibodies were similar in J5 antiserum and control serum (Baumgartner *et al.*, 1991). Since the mechanism of protection remains obscure, it is difficult to explain why some clinical studies have given positive results and others have not.

In the study of the E5 mAb (Greenman *et al.*, 1991), 486 patients with a septic syndrome believed to result from Gram-negative infection, were randomly assigned to receive this antibody or an identical volume of saline. No decrease in mortality was observed in the overall group of 468 assessable patients nor in the 316 patients who subsequently proved to have a documented Gram-negative infection. Among these 316 patients, there was a statistically significant decrease in mortality in 137

patients without shock at trial entry ($P = 0.03$, Kaplan-Meier), but 179 patients with shock were not protected. A second multicentre study of 931 patients, specifically designed to verify these results, failed to confirm that E5 was able to improve the survival of patients with Gram-negative septic syndromes without shock (Wenzel *et al.*, 1991).

The second antibody, HA-1A, was studied in 607 patients with a presumptive diagnosis of Gram-negative sepsis, among whom 543 were evaluated (Ziegler *et al.*, 1991). Among the 401 patients with Gram-negative infections, the sepsis syndrome was definitely attributed to Gram-negative bacteria if blood cultures were positive for a Gram-negative bacteria (200 patients), probably if negative blood cultures were felt to be due to the presence of antibacterial agents (117 patients), and possibly if blood cultures were negative despite the absence of an effective antibacterial agent (84 patients). HA-1A did not reduce the mortality at 28 days in the overall study population (43% in the placebo recipients and 39% in the HA-1A recipients), nor did it reduce mortality in the 142 patients without documented Gram-negative infections. Furthermore it failed to reduce mortality, even marginally, in the 401 patients with Gram-negative infections, as well as the 117 patients with probable Gram-negative sepsis, and the 84 patients with possible Gram-negative sepsis. However, there was a decrease in mortality in the 200 patients with Gram-negative bacteraemia (49% (mAb) and 30% (placebo), respectively, $P = 0.014$). The difference was more significant among the 101 patients who were in shock than among the 99 patients not in shock.

These data reveal that the selection of the patients who might benefit from this treatment will present a major problem. The mortality rate was identical in HA-1A and placebo recipients among all patients treated and in patients with non-bacteraemic Gram-negative sepsis syndromes. The clinical trial of HA-1A was designed to select as precisely as possible patients with a septic syndrome due to Gram-negative infections. At present there is no rapid diagnostic test which allows more precise selection of the subset of patients presenting with Gram-negative bacteraemia. To await the results of blood culture does not seem realistic. Since HA-1A costs £2200 (plus Value Added Tax) per single dose, some medical economists have indicated that the cost of this treatment, if widely adopted, could absorb almost 20% of the current expenditure on

drugs by NHS hospitals in the United Kingdom (Taylor, 1991).

Detailed analysis of the study reveals another cause for concern. There may have been imbalances in risk factors at randomization between placebo and HA-1A recipients in the subgroup of 200 patients with Gram-negative bacteraemia (Carlet *et al.*, 1991); in table 4 of the published study (Ziegler *et al.*, 1991), it can be calculated that a total of 101 serious complications (disseminated intravascular coagulation, adult respiratory distress syndrome, acute hepatic failure and acute renal failure) were observed at entry among the 95 placebo recipients (a mean of 1.06 per patient) compared to 85 among the 105 HA-1A recipients (0.81 per patient). Although a Cox proportional-hazards model revealed that the difference remained significant among both severely ill (APACHE II score > 25) and less severely ill patients (APACHE II score ≤ 25) (Ziegler *et al.*, 1991), these 16 additional serious complications in the placebo group might nevertheless account for some of the higher mortality in this group of patients (13 excess deaths).

Since our basic understanding of the specificity and of the function of HA-1A is incomplete; since animal protection studies have given discrepant results; since other clinical studies with similar anti-LPS antibodies have failed to show benefit, the possible imbalance between the test and controlled groups at entry in the HA-1A study suggests that it may be premature to rely on the single clinical HA-1A study before widely adopting this novel therapeutic approach to sepsis. Despite the present urgent need for adjunctive therapy of Gram-negative septic shock, the use of antibodies directed at lipid A or at other epitopes of the core LPS should still be considered investigational. Clearly, further basic research is needed to clarify the protective mechanism of such antibodies in advance of further clinical trials.

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